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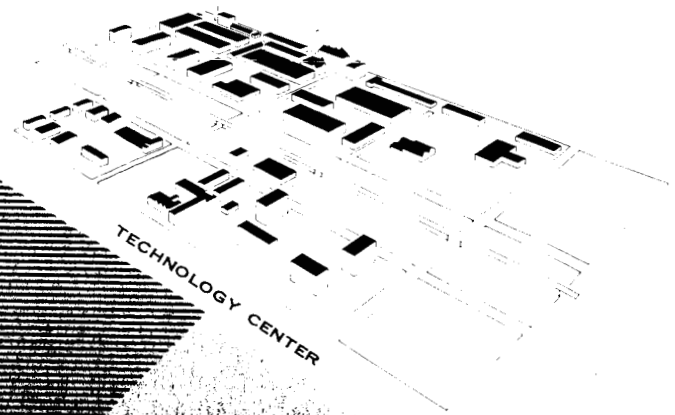

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Report No. ARF C 194-8
(Phase Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

National Aeronautics and Space Administration
Washington, D. C.

ARMOUR RESEARCH FOUNDATION
of
Illinois Institute of Technology
Technology Center
Chicago 16, Illinois

ARF Project C 194
Contract No. NASr-22

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Report No. ARF 3194-8
(Phase Report)

Charles A. Hagen and Ervin J. Hawrylewicz

for

National Aeronautics and Space Administration
Washington, D. C.

Copy No._____

February 28, 1963

FOREWORD

This is Report No. ARF 3194-8 (Phase Report) on Contract NASr-22, entitled "Life in Extraterrestrial Environments." The report covers the period from February 15, 1962, to February 28, 1963. The results of experiments conducted during the first three quarters of this period have been previously presented in detail and are only summarized in this report.

Dr. R. E. Cameron, of the Jet Propulsion Laboratory, California Institute of Technology, supplied the desert soil samples. Dr. P. Ponce de Leon, of the Chicago Natural History Museum, assisted in the collection and identification of the lichens. Mr. K. A. Basa conducted some of the experiments. Technical assistance was given by Mr. J. Rush, Mr. P. Barbera, and Mr. L. Pollard.

The experimental data are contained in ARF Logbooks C 11194, C 11439, C 11543, C 11639, C 11724, C 12026, C 12033, C 12112, C 12271, C 12508, C 12706, C 12859, and C 13027.

Respectfully submitted,

ARMOUR RESEARCH FOUNDATION
of Illinois Institute of Technology

Charles A. Hagen
Associate Bacteriologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Manager
Life Sciences Research

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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

I. INTRODUCTION

The objective of this program is to study the effect of a simulated Martian environment on the survival of terrestrial microorganisms and plants.

The results of previous experiments are described in detail in ARF Reports 3194-2, -3, -4, -5, -6, and -7 and are summarized in the following paragraphs.

Lichens of the genera Cladonia, Parmelia, Physica, Umbilicaria, Lecidea, and Ramalina were subjected to a simulated Martian environment for 2 months. Microscopic examination of the algal portion of these lichens indicated that the lichens had not survived.

Pure cultures of Bacillus licheniformis ATCC 8480, B. megaterium ATCC 10778, B. brevis ATCC 8185, B. cereus ATCC 2, and B. subtilis var. globigii ARF culture collection were studied for survival in the simulated Martian environment. Some of the Bacillus spp. were capable of surviving in the simulated Martian environment, but growth was not demonstrated. B. cereus did not survive a 28-day test period. Within the same time period the number of B. megaterium cells did not change significantly from the initial number. B. licheniformis and B. brevis showed 42 and 25% survival after 28 days, respectively. After 120 days the mean value of the number of B. subtilis cells was not significantly different from the initial mean value.

Further studies with the B. subtilis strain did not show evidence of adaptation to the Martian environment. In these adaptation studies, only total cell

counts were made and no attempt was made to determine cellular biochemical changes or the relative percentages of dead, injured, and uninjured cells. The number of B. subtilis cells surviving was related to the number of spores present. A cyclic increase-decrease survival pattern was observed, with maximal peaks at 1 and 28 days of exposure to the Martian environment.

Studies with bacterial cells indicated that the simulated Martian atmosphere and soil had a toxic effect on the cells. Exploratory experiments showed that addition of organic medium and moisture significantly affected the survival of bacterial cells. The number of cells increased more than 3000% during a 28-day test period when 0.18% water (v/v) and 10% organic medium (w/w) were present even though the Martian diurnal temperature cycle was imposed.

Studies of the effect of the Martian environment upon the native flora of California desert soil also presented evidence that microorganisms can survive the simulated Martian environment. A total of 235 cultures was isolated from the various desert soil samples before and after exposure to the simulated Martian environment for 28 and 84 days. On the basis of colonial morphology and Gram's stain reaction, the predominating microorganisms present were Bacillus spp. with moderate numbers of Actinomyces spp. and Micrococcus spp. Molds were also present in several of the desert soil samples.

At present time two cultures of Coccus spp. and two cultures of Bacillus spp. cultures are being kept under the Martian environment as part of the survival studies. Of these, the Coccus sp.. isolated from a sandy loam collected from

the Colorado desert 5 miles east of Thermal, California, has not decreased in numbers over a 7-day test period.

During the current period survival experiments were performed with two strains of Aerobacter aerogenes: ATCC 13048 and the butanediol-producing culture ATCC 8724. The objective was to determine whether 2, 3-butanediol protects the cells during the freezing-and-thawing cycle experienced in the Martian environment.

At the end of the 28 day test period less than 1% of the Aerobacter cells survived. No significant difference in survival rate was noticed between the strains.

II. EXPERIMENTAL WORK

The simulated Martian atmosphere previously described in Report ARF 3194-5 was used. The methods of growing, harvesting, inoculating, and sampling the Mars tubes and the types of media used are described in detail in Reports 3194-2, -3, -4, -6, and 7.

A. Aerobacter aerogenes

The Aerobacter cultures were obtained from the American Type Culture Collection, Washington, D.C. They were A. aerogenes ATCC 13048 and ATCC 8424; strain 8724 produces 2, 3-butanediol.

The control tubes, containing 1 g of simulated Martian soil (equal parts by weight of limonite and felsite), were inoculated with 0.01 ml of A. aerogenes culture, evacuated, and flushed seven times with earth atmosphere. The tubes were then sealed at 760 mm Hg pressure. The Martian experimental tubes were treated in the same way except that the simulated Martian

atmosphere (93.8% nitrogen, 4% argon, 2.2% carbon dioxide) was used for flushing. After seven evaculations and flushings the tubes were sealed at 85 mm Hg pressure. The Martian experimental tubes were subjected to a diurnal temperature cycle of + 26° C to -60° C throughout the test period. The control tubes were kept at 26° C throughout the test period. After exposure periods of 0, 1, 7, and 28 days three tubes from each group were opened and quadruplicate platings at each dilution were performed on Difco plate count agar using 0.1% peptone water as the diluent. The plates were incubated at 35° C for 48 hours. Cumulative totals from two separate experiments (a total of six tubes, or 24 plates from each group) were used to calculate the percent survival.

B. Desert Soils

The desert soil samples were kept in screw-capped test tubes at room temperature. Plate counts to determine population changes were made after 1 and 7 months of storage.

Mars tubes containing 1-g portions of the desert soil samples were flushed with the Martian atmosphere, sealed at 85 mm Hg pressure, and plated on agar after exposure to the diurnal temperature cycle for 1, 28, and 84 days. Conventional control samples were included in these experiments. Representative colonies were picked from the plates. The isolates were initially transferred into Difco nutrient broth with 0.5% glucose added. The cultures were then inoculated onto agar slants and stored in a refrigerator. During the isolation procedures colonial morphology on agar, Gram's stain reaction, and growth characteristics in nutrient broth were recorded. Further tests to determine species will be performed on selected cultures as necessary.

At present, four cultures isolated from desert soil samples kept in the Martian environment for 84 days are being studied. These cultures are: a Gram-positive Coccus sp. and a Bacillus sp. from the sandy loam sample collected 5 miles east of Thermal, California; and a Gram-positive Coccus sp. and a Bacillus sp. from a clay sample collected 15 feet from the sandy loam sample. These two Bacillus samples were chosen for these initial studies because results from previous experiments showed significant increases in the total number of bacteria without an increase in the number of spores.

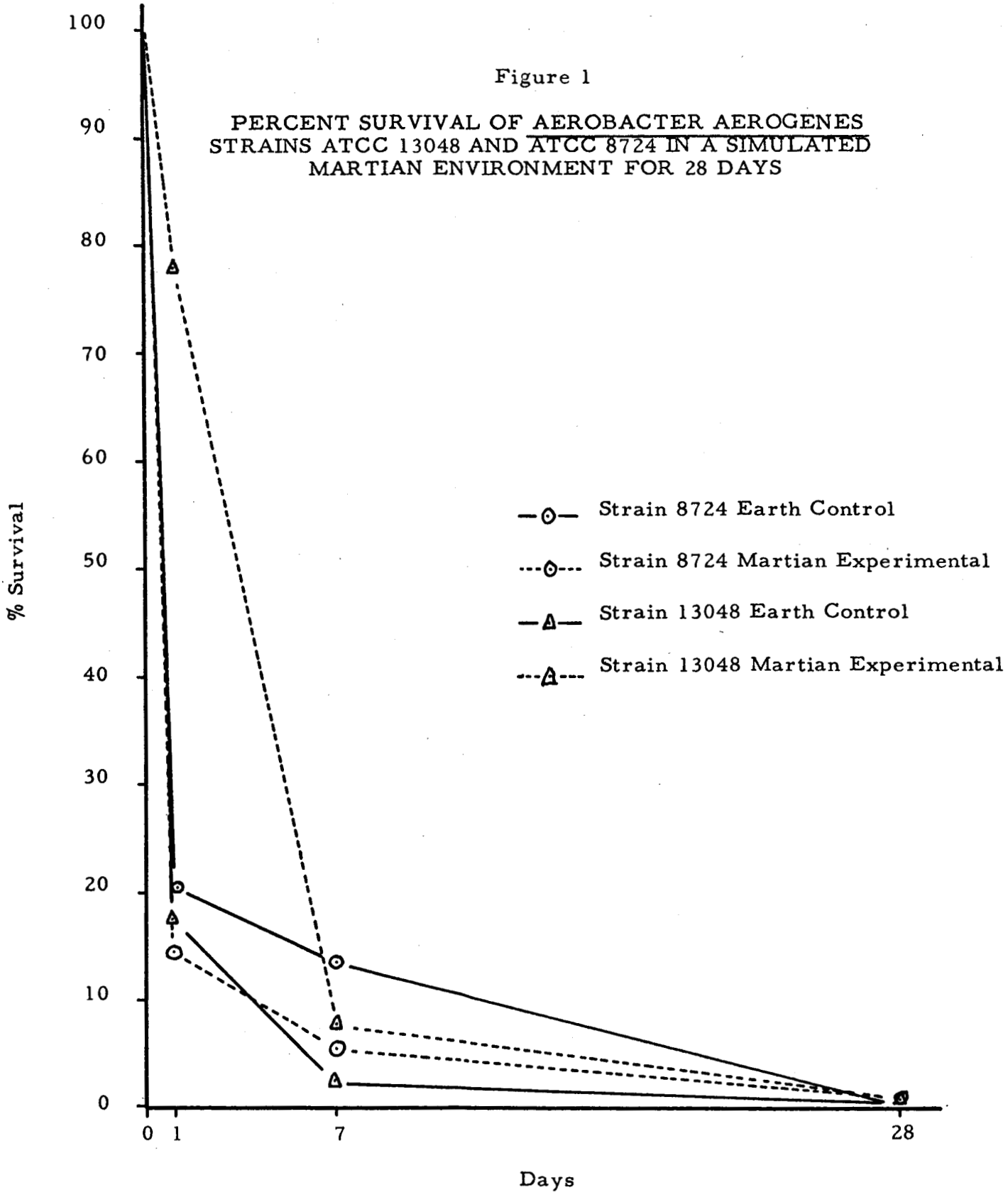
III. RESULTS AND DISCUSSION

A. Aerobacter aerogenes

Figure 1 shows the survival curves of A. aerogenes during a 28-day test period. At the end of 1 day the butanediol-producing culture (ATCC 8724) had 21 and 15% of the initial cell population surviving in the control and Martian experimental groups, respectively. At the end of 28 days less than 1% of the cells was recovered from either group. After 1 day the ATCC 13048 culture had 18 and 78% of the initial cell populations surviving in the control and Martian experimental groups, respectively. It was surprising to find such a high survival in the experimental group after a freezing-thawing cycle. Usually, the population of a Gram-negative microorganism like Aerobacter decreases 90% or more in a single freezing-thawing cycle. However, the number of cells surviving in the experimental group decreased to 8% after 7 days, and less than 1% of the cells survived the 28-day test period in either the control or Martian groups.

Figure 1

PERCENT SURVIVAL OF AEROBACTER AEROGENES STRAINS ATCC 13048 AND ATCC 8724 IN A SIMULATED MARTIAN ENVIRONMENT FOR 28 DAYS



B. Desert Soil

A population change occurred in the desert soil samples kept at room temperature in tightly stoppered screw-capped test tubes. Initially the Bacillus spp. were the predominating microorganisms, with smaller to equal numbers of Actinomyces spp. present. After 6 months the number of Actinomyces decreased significantly. In three of the samples, No. 51, 62, and 68, the number of bacteria increased after 6 months.

The pH, moisture, organic content, temperature, and symbiotic and antagonistic relationships among the microorganisms are but a few of the variables that must be established in order to explain the population changes. Present efforts are concerned with isolating organisms from soils exposed to extreme environmental conditions, i.e., low temperatures, large temperature variations, and reduced pressures, and with determining their ability to survive in the simulated Martian environment. More detailed studies designed to elucidate the mechanism of survival will be conducted with the surviving cultures.

Although the experimental data of the four isolates from desert soil are of a preliminary nature, a few facts are apparent. The two Coccus cultures withstood the inoculation and flushing operations better than the two Bacillus cultures: 11 and 22% of the Coccus cells from samples No. 62 and 68, respectively, survived, compared with 0.1 and 0.04% of the Bacillus cells isolated from the same samples. After 7 days in the Martian environment the number of Coccus organisms in sample No. 62 had not decreased. The average number of viable cells initially present was 35×10^4/ g. The average number of cells present after 7 days was 54×10^4/ g. The heat stability

of the spores from both Bacillus cultures was poor. The spores withstood 60° C but not 80° C for 10 minutes.

IV. SUMMARY

Two Aerobacter cultures, ATCC 13048 and butanediol-producing ATCC 8724, were subjected to simulated Martian environment for 28 days to determine their survival. At the end of 28 days less than 1% of the cells survived in either of the cultures.

A total of 235 representative microorganisms was isolated from five desert soil samples. The isolates were recovered from the samples before exposure to the Martian environment and after exposure for 28 and 84 days. Colonial morphology on agar, growth characteristics in nutrient broth, and the Gram's stain reaction of the isolates were recorded. Members of the genus Bacillus were most frequently present in the soil samples. Actinomyces organisms were present in fewer numbers, and ocasionally Micrococcus and molds were present. With increased time in the Martian environment there was a decrease in the number of Actinomyces.

Studies were made with two pure Coccus and two pure Bacillus cultures isolated from the desert soil samples in contact with the Martian environment for 84 days. The Coccus cultures were more resistant to inoculation-flushing procedures and subsequently to the simulated Martian environment than the Bacillus cultures.

V. FUTURE PLANS

The future program will include:

A. Survival, adaptation, and growth studies of microorganisms

B. Survival, adaptation, and growth studies of plants

C. Biochemical studies relating to growth of plants and microorganisms

D. Physical studies in support of the growth experiments.

Initial data on desert soil samples in the Martian environment suggest that soils from other geological and geographical locations be examined.

The isolation, screening, and adaptation studies of organisms obtained from these soils will be continued. Microorganisms exhibiting high survival rates in the Martian environment will be studied biochemically in an attempt to define the mechanism of survival.

Studies with pure cultures of microorganisms will be continued and will include autotrophs and heterotrophs.

Studies with plants will utilize experimental procedures similar to those planned for the microbiological phase. The screening studies of algae, bryophytes, and lichens carried out in the initial phases of the program will be extended. Efforts are now being made to obtain flora from cold environments and elevated altitudes. Xeromorphic plants will be included in these studies.

Studies will be conducted to establish whether any metabolic or structural changes occur in microorganisms or plants surviving the Martian environment. These include changes in respiration rate, enzyme composition,

growth rate, and morphological characteristics as a function of environmental change. Physical measurements in support of the growth experiments will involve measurements of exceedingly low vapor pressures, soil analyses, and definition of the effect of the freezing rate (ice crystal formation) on survival of microorganisms and plants.

A detailed discussion of these proposed studies was presented in ARF Proposal No. 63-414 L, dated December 28, 1962.

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